Monitoring Overeruption Pattern of Young vs Adult Unopposed Molars in the Rat

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Purpose: To investigate the overeruption of unopposed molars and the adaptation of alveolar bone height in young and adult rats. Materials and Methods: A total of 56 4-week-old (young) and 26-week-old (adult) male Wistar rats were followed up longitudinally. In each age group (n = 28), 16 rats were included in the experimental subgroup, in which all the maxillary right molars were extracted, and 12 rats were included in the control subgroup. All rats were scanned at regular intervals with in vivo microcomputed tomography for a 12-week period, and the eruption rate of the mandibular first molars and the surrounding bone were measured, with the reference point at the mandibular canal. The molar categories were unopposed right molars and overloaded left molars in the experimental group and control molars in the control group. Results: The young unopposed molars had the highest mean eruption rate (172 ± 67 µm/day). The overeruption was most marked during the first 3 weeks postextraction, thereafter gradually returning to a level comparable to controls (12.9 ± 6.8 µm/day). The overloaded adult molars did not erupt during the experimental period. Alveolar bone height also increased in young unopposed molars, but at a slower pace than overeruption, causing an increase in clinical crown height. Conclusion: Unopposed young molars overerupt at a higher pace than adult molars during the first weeks postextraction. The alveolar bone grows in response to the tooth eruption but to a lesser extent, which leads to a constant increase in clinical crown height at the same rate in young and adult rats. The increase of clinical crown height was 4.5-fold higher in young unopposed molars compared to control molars. Int J Prosthodont 2020;33:663–670. doi: 10.11607/ijp.6644

P osteemergent eruption takes place starting from the moment the tooth pierces the gingiva and continues throughout the tooth’s lifetime.1,2 Initially, this eruption brings the tooth into occlusion with its antagonist (preocclusal phase) and afterwards maintains functional contact in order to compensate for craniofacial growth and occlusal wear (functional phase). When the antagonist tooth is lost, the unopposed tooth will overerupt in an effort to find occlusal contact (postocclusal phase), which may lead to severe overeruption.3

Anthropologic studies have characterized the continuous eruption that occurs during the functional phase as a physiologic mechanism to compensate for tooth attrition.4–8 Most studies have used the inferior alveolar nerve canal as a stable reference point and have estimated tooth eruption using the cementoenamel junction (CEJ) or the apex. It has been observed that both of these structures move further away from the canal, and this movement was positively correlated with the amount of occlusal wear.4–8

In modern populations, dental implants show continuous eruption via the vertical discrepancy between adjacent teeth and the implant crown over periods of several years.9–11 Initially thought to be mainly present in adolescents, functional eruption has been shown to occur at all ages12,13 to different degrees. It has been shown that functional eruption decreases with age, but also that there is a high variability between individuals, and thus that the degree of eruption cannot be readily identified by simple factors such as age or gender.12 Thus, although the recommended age for implant placement has increased, even implants placed during adulthood can develop relative
infraocclusion after a few years and cause problems regarding treatment planning and outcome.

Furthermore, the postocclusal phase of eruption has a higher eruption rate than the functional phase and is often termed “overeruption” because the unopposed tooth erupts past the occlusal plane in search of occlusal contact. There is also a significant amount of variability in the amount of overeruption among individuals with unopposed molars. It was found that 75% had none or minimal overeruption, while 25% showed moderate to severe overeruption, with more pronounced overeruption of unopposed molars in young adults. These findings demonstrate the complex clinical decision-making regarding patients with unopposed teeth and the time frame for prosthodontic treatment.

In animal experiments, overeruption after a 4-week period was found to be more significant if the antagonist tooth was lost early in life, in childhood, or in adolescence compared to adulthood. To better understand the eruption patterns of these different stages of tooth eruption, this study undertook an in vivo microcomputed tomography (microCT) study in rat molars. The aim of the present study was to document the eruption pattern of unopposed molars in rats, as well as their overloaded contralateral molars, in relation to the surrounding alveolar bone in a longitudinal study design.

Materials and Methods

Sample Size Calculation

The effect size was set at double the normal eruption rate (23 µm/day), the power at 90%, the α at 5%, and the SD at 18.5 µm/day, based on a previous study. The sampling ratio was set at 0.75 because the left and right molars in the experimental rats served two different experimental subgroups, whereas both sets of molars in the control rats served as control molars.

Animals

A total of 56 Wistar male rats were split into two age groups (n = 28), 4 weeks and 26 weeks (young and adult, respectively), and followed longitudinally using microCT. The animals were housed 2 to 4 per cage, depending on their weight. Each age group was divided into two subgroups: experimental (n = 16) and control (n = 12).

At the beginning of the study, the maxillary right molars were extracted in the experimental group. Extraction was performed under anesthesia by intraperitoneal injection of ketamine 90 mg/kg and xylazine 10 mg/kg and was supplemented by buprenorphine (0.1 mg/kg sc) 15 minutes before the surgery and by postoperative paracetamol (Dafalgan, UPSA) solution (2 mg/mL) for 48 hours after the surgery. The teeth were disinfected with chlorhexidine 0.2% before extraction, and an extraction plier was used to extract the teeth. For the duration of the experiment, the animals were fed a soft diet and had water ad libitum. The body weight of the animals was measured every time the animals were scanned to monitor their condition. Two control rats died during the scanning procedure. This study was approved by the ethics committee of animal research under the number GE/84/17. This research follows the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines.

MicroCT Investigations

All rats were followed up for 12 weeks and scanned in the morning at regular intervals with the Quantum GX microCT system (PerkinElmer). The rats were scanned 3 times per week during the first week, 2 times per week during the next 2 weeks, and then once per week until the end of the experimental period. The choice of scanning frequency was based on the hypothesis that unopposed molars would initially erupt faster and then decrease in velocity over time. Based on the observed velocities in previous studies, it was estimated that the initial 3 times per week would be sufficient to detect changes of this magnitude. Isoflurane anesthetic gas was used during the scans. The gas was administered at 5% and 1 L/minute for the induction phase and at 3% and 1 L/minute during the scans. The scan resolution was set at 30 µm.

Morphometric Analysis

The 3D images were exported to OsiriX image analysis software. Three mandibular molar categories were studied: unopposed (after extraction of the antagonist), overloaded (contralateral to the unopposed), and control. Mandibular first molars were measured at three locations (the first, second, and third roots) on coronal plane slices. The mandibular canal was used as a stable landmark. The tips of the lingual and buccal cusps and the alveolar bone crest were used to measure tooth position and bone height. The molar position and the alveolar bone height were calculated as the mean of the lingual and buccal distances to the center of the mandibular canal (Fig 1). The daily eruption velocity was obtained by calculating the difference between two subsequent tooth position measurements divided by the number of days between the measurements. Alveolar bone height measurements were used to calculate the rate of clinical crown height increase (ie, the difference between the alveolar bone height and the cusp height). Unopposed molar eruption velocity was evaluated in three phases over the course of the experiment: (1) 0 to 3 weeks, (2) 4 to 7 weeks, and (3) 8 to 12 weeks.

Statistics

SPSS statistical program (IBM) was used for all tests. Weight comparisons between the control and experimental rats were performed separately for young and adult rats using unpaired t test. Normal distribution
of data was visually assessed on a histogram overlaid with a normal distribution curve. Molar eruption was analyzed using 3-way ANOVA with the dependent variables cusp height, alveolar bone height, cusp vertical displacement, and clinical crown height, and the independent variables age group (young, adult), molar category (control, unopposed, overloaded), and experimental phase (0 to 3 weeks, 4 to 7 weeks, and 8 to 12 weeks). Tukey post hoc analysis was performed to compare conditions for the variables molar category and experimental phase.

Method Error
Possible measurement error was assessed by 60 repeated measurements of the molar position 1 week apart by the same operator (A.L.). The repeated measures were compared using paired \( t \) test for systematic error, and the Dahlberg formula\(^ {23} \) was used to estimate random error.

**RESULTS**

**Body Weight**
No significant differences were found between the experimental and control groups regarding mean body weight.

**Method Error**
No significant systematic error was found for the cusp or alveolar bone height measurements. The random error was 33.4 µm and 33.5 µm for
the cusp and alveolar bone heights, respectively.

Molar Eruption and Alveolar Bone Level

All molar categories erupted continuously throughout the experimental period, with the exception of the adult overloaded molars, which showed no signs of eruption (Fig 2a). The total overeruption of unopposed molars was much higher in young rats than in adult rats, and by day 84, these molars had reached the occlusal height of the adult control molars. Furthermore, the adult unopposed molars did not erupt more than the adult control molars. Similarly, the young overloaded molars erupted similarly to control molars, but adult overloaded molars showed no eruption. Alveolar bone growth followed tooth eruption, but not at the same rate (Fig 2b). The difference between molar eruption velocity and alveolar bone growth was particularly high in the young unopposed molars, with the alveolar bone growth only slightly increased with respect to the amount of molar overeruption. In adult rats, all molar categories showed a very limited increase in alveolar bone height.

Eruption Velocity

The mean eruption velocity of molars in young rats was higher than in adults (Fig 3). In young rats, the unopposed molars erupted significantly faster than the control and overloaded molars (Table 1a). On the contrary, in adult rats, unopposed and control molars showed no difference, whereas overloaded molars had a significantly lower eruption rate. On the first day postextraction in the young unopposed molars, the mean peak velocity was 172 ± 67 µm/day. Overloaded molars in young and adult rats showed no signs of eruption on day 3 postextraction, with an average eruption velocity of –14 ± 46 µm/day.

The experiment was divided into three periods for analysis (Fig 4). Eruption velocity in the three experimental phases was statistically different (P ≤ .003), decreasing from first to third. All molars except for the overloaded adult molars showed a reduction in eruption velocity between the first and third experimental phases (Table 1b). Unopposed molars showed the highest change in eruption velocity, followed by the control and then the overloaded molars.

Clinical Crown Height

Initially, young rats had a smaller clinical crown height than adult rats (Fig 5). The young unopposed molars had a 4.5-fold—higher increase in clinical crown height compared to the control molars. All molars showed an increase in clinical crown height throughout the experimental period except for the overloaded adult molars (Fig 6 and Table 2).
Table 1a  Comparison of Mean ± SD Eruption Velocity (μm/d) Among Molar Groups

<table>
<thead>
<tr>
<th></th>
<th>Control (C)</th>
<th>Unopposed (U)</th>
<th>Overloaded (O)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>16.2 ± 7.4</td>
<td>35.4 ± 25.6</td>
<td>17.1 ± 6.9</td>
<td>.000</td>
</tr>
<tr>
<td>Adult</td>
<td>6.2 ± 4.1</td>
<td>7.8 ± 8.3</td>
<td>2.0 ± 1.7</td>
<td>.868</td>
</tr>
</tbody>
</table>

P values were obtained using Tukey post hoc tests.

Table 1b  Comparison of Mean ± SD Eruption Velocity (μm/d) Among Experimental Phases

<table>
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<tr>
<th></th>
<th>Phase 1: 0–3 wk</th>
<th>Phase 2: 4–7 wk</th>
<th>Phase 3: 8–12 wk</th>
<th>1 vs 2</th>
<th>1 vs 3</th>
<th>2 vs 3</th>
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<tbody>
<tr>
<td>Young</td>
<td>Control</td>
<td>23.0 ± 6.3</td>
<td>17.4 ± 2.7</td>
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<td>.000</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Unopposed</td>
<td>66.9 ± 15.8</td>
<td>26.6 ± 4.5</td>
<td>.000</td>
<td>.000</td>
<td>.000</td>
</tr>
<tr>
<td></td>
<td>Overloaded</td>
<td>22.9 ± 5.5</td>
<td>18.8 ± 3.0</td>
<td>.012</td>
<td>.000</td>
<td>.000</td>
</tr>
<tr>
<td>Adult</td>
<td>Control</td>
<td>10.5 ± 3.9</td>
<td>5.4 ± 1.4</td>
<td>.001</td>
<td>.000</td>
<td>.077</td>
</tr>
<tr>
<td></td>
<td>Unopposed</td>
<td>13.7 ± 11.1</td>
<td>6.5 ± 4.9</td>
<td>.019</td>
<td>.000</td>
<td>.427</td>
</tr>
<tr>
<td></td>
<td>Overloaded</td>
<td>2.3 ± 2.5</td>
<td>2.0 ± 1.3</td>
<td>.889</td>
<td>.750</td>
<td>.966</td>
</tr>
</tbody>
</table>

Fig 5  Changes in clinical crown height among molar categories and age groups over the course of the study.

Fig 6  Mean clinical crown height compared among molar categories and age groups. P values were obtained from Tukey post hoc multiple comparisons. *P < .05. **P < .001.
DISCUSSION

The present study showed that the three factors studied—occlusal loading, animal age, and time after loss of the antagonist tooth—had a statistically significant influence on molar eruption velocity. Young rats showed a higher eruption velocity than adult rats in all molar categories (ie, control, unopposed, and overloaded). Similar results were observed for alveolar bone growth, although the overall rate was less than the eruption velocity.

The present findings are in line with the literature, in which young unopposed teeth are found to have more overeruption compared to adult teeth, although Fujita et al.21 observed slightly less overeruption. This difference might be due to their method of creating the unopposed molars; Fujita et al ground the antagonist teeth instead of extracting them, which prevented occlusal contact but potentially allowed for bigger food particles to transfer occlusal forces to the unopposed teeth. A second difference is that these authors found a statistically significant increase in eruption in the unopposed adult group compared to the control group, although our results showed a decrease in eruption in the unopposed adult group. A second difference is that these authors found a statistically significant increase in eruption in the unopposed adult group compared to the control group, although our results showed a decrease in eruption in the unopposed adult group.

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The present results suggest there is an equilibrium between occlusal forces and tooth eruption potential that determines the amount of functional and postocclusal eruption. The eruption potential seems to have a buffer capacity that can compensate for the increased loading seen in overloaded young molars. The difference in overeruption between young and adult unopposed molars could result from an age-dependent decrease of this eruption potential. Such a decrease would also explain the lack of eruption of overloaded adult molars, where the eruption potential is no longer sufficient to compensate for the increased occlusal loading.

To the best of the authors’ knowledge, no studies have used a longitudinal approach to examine unopposed eruption over a period of several months. The first 3 days showed the most significant overeruption in the young unopposed molars, which decreased rapidly during the first phase (0 to 3 weeks). Although this study presents evidence of different phases of overeruption that resemble the clinical environment, human studies are required in order to confirm the importance of these periods and to define their lengths.

The eruption velocity graph (Fig 3) showed that both unopposed and overloaded young molars had increased eruption in the first days, with eruption of the unopposed molars considerably higher. In line with this finding, Dorotheou et al.24 showed increased cellular activity in the periodontal ligament in the young unopposed and overloaded molars 3 days after extraction, with eruption in the unopposed molars higher than the overloaded molars. The hypothesis for this temporary increase—rather than decrease—of velocity in overloaded molars is that, following the intervention, the animals may have...
had reduced masticatory function due to postextraction discomfort. The expression of alpha smooth muscle actin (α-SMA), a marker of myofibroblast cells that are thought to participate in tooth eruption, was increased after 3 days compared to after 15 days and compared to control molars.25 Furthermore, differential expression of α-SMA was not observed in adult unopposed molars, which is in line with the lack of overeruption seen in this study and suggests that myofibroblasts play an important role in tooth eruption potential.

The clinical crown height (the cusp height minus the alveolar bone crest height) was calculated to illustrate the relationship between these two tissues. An increase of 242 μm over the 12-week experimental period was observed, with no difference between young and adult control molars. Hoffman and Schour26 showed a clinical crown height increase, as measured between the CEJ and the alveolar bone crest, of approximately 600 μm over a 12-week period starting at day 28, but also observed 260 μm of tooth wear due to a hard diet, which contributes to increasing the distance between the alveolar bone and the CEJ by compensatory eruption. Therefore, the present results closely resemble theirs, and the same conclusion was made: that tooth eruption rate exceeds alveolar bone growth rate.

Surprisingly, the clinical crown height was not constant among all molar categories, but was influenced by masticatory function. The young unopposed molars showed a very important increase in clinical crown height, surpassing the adult control molars by far. Studies have suggested that decreased bone activity seen with increased age27–29 can be the cause of reduced unopposed tooth eruption in adults compared to young individuals.21 While differences in eruption velocities of young and adult unopposed teeth were observed, there was no evident link to alveolar bone growth rate. On the contrary, in the young unopposed molars, the bone appeared to have no control over tooth eruption, as the clinical crown height gain was increased 4.5-fold compared to the control molars. In the present study, the main factors governing tooth eruption velocity were the level of occlusal force and age. The present authors suggest that age is a limiting factor not through alveolar bone growth, but through a decrease of the intrinsic eruptive potential of the tooth.

In humans, overeruption occurs in varying degrees, and one of the influencing factors is age—more specifically, young individuals are more susceptible to overeruption than adults.20 However, several human studies in adults have found a significant overeruption of unopposed teeth compared to controls.14,17,19,20 Although differences were not observed between adult unopposed and control molars in the present study, there was a difference between unopposed and overloaded molars. The studies that have found significant overeruption in adult molars used occluded teeth and control molars from the same patient, a confounding factor that does not allow for differentiation between unopposed and overloaded situations. Similarly to Fujita et al.,21 these studies may have measured a relative overeruption. As seen in the adult overloaded molars, the effect of occlusal loading on teeth has been indirectly shown in humans via masseter muscle thickness. Rohila et al.30 found a negative correlation between masseter muscle thickness and vertical facial pattern; ie, hyperdivergent individuals showed thinner muscle than hypodivergent individuals, indicating that higher occlusal forces may lead to less eruption of teeth. In line with the present results, another study found that clinical crown height in humans increased during adolescence and young adulthood.31 Overall, the rat model gives good insight into the tooth eruption and alveolar bone dynamic; however, as the height of the rat mandible is proportionally smaller than the human mandible, there might be differences between the present findings and a human clinical environment.

In the present experiment, unilateral molar extraction was performed by fully removing the tooth instead of grinding the crown, which ensures that molars remain unopposed during the experimental period. As lack of teeth could affect the chewing ability of experimental rats, both the experimental and control groups were fed a soft diet instead of the normal hard diet. No significant differences were found in body weight between the experimental and control groups, showing that the well-being of the animals was not influenced by the reduced masticatory capacity. While a soft diet ensured similar dietary conditions between the control and experimental groups, it could also have increased the rate of eruption of the control molars. If indeed the eruption was increased, this may have affected the measured increase in clinical crown height. The technique used to measure alveolar bone height also affected the clinical crown height. This measurement is slightly biased because the distances measured are not parallel to the eruption path; instead, they are at a slight angle because the mandibular canal is in the center of the tooth and the alveolar crest is on the sides. The measured alveolar bone height gain is smaller than the actual gain; however, these are potential systematic errors that were present in all molar categories and age groups and thus do not affect the comparison among groups. The random method error of this study applies to the cusp and alveolar bone height measurements, but it cannot be compared directly to the computed velocities because those values are per day, not per measurement, which were several days apart. In relation to the cusp and alveolar bone height measurements, the random error is low.
CONCLUSIONS

The young unopposed molars overerupted mainly during the first 3 weeks. In the adult experimental group, a relative overeruption was observed due to decreased eruption of the overloaded molar, instead of overeruption of the unopposed molar. The alveolar bone height increased with tooth eruption, although at a slower pace than molar eruption. This difference resulted in an increase of the clinical crown height in all molar categories, with the exception of the adult overloaded molars. The young unopposed molars showed a significantly higher gain in clinical crown height compared to controls.

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